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The evolution of plasmid stability: Are infectious transmission and compensatory evolution competing evolutionary trajectories?

James P. J. Hall[1], Michael A. Brockhurst[1], Calvin Dytham[2] & Ellie Harrison[1]

[1]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK

[2]Department of Biology, University of York, York, YO10 5DD, UK

Abstract

Conjugative plasmids are widespread and play an important role in bacterial evolution by accelerating adaptation through horizontal gene transfer. However, explaining the long-term stability of plasmids remains challenging because segregational loss and the costs of plasmid carriage should drive the loss of plasmids though purifying selection. Theoretical and experimental studies suggest two key evolutionary routes to plasmid stability: First, the evolution of high conjugation rates would allow plasmids to survive through horizontal transmission as infectious agents, and second, compensatory evolution to ameliorate the cost of plasmid carriage can weaken purifying selection against plasmids. How these two evolutionary strategies for plasmid stability interact is unclear. Here, we summarise the literature on the evolution of plasmid stability and then use individual based modelling to investigate the evolutionary interplay between the evolution of plasmid conjugation rate and cost amelioration. We find that, individually, both strategies promote plasmid stability, and that they act together to increase the likelihood of plasmid survival. However, due to the inherent costs of increasing conjugation rate, particularly where conjugation is unlikely to be successful, our model predicts that amelioration is the more likely long-term solution to evolving stable bacteria-plasmid associations. Our model therefore suggests that bacteria-plasmid relationships should evolve towards lower plasmid costs that may forestall the evolution of highly conjugative, 'infectious' plasmids.

Main

Plasmids, and the vast pool of bacterial accessory genes they carry, are both a substantial source of genetic material for bacteria, and agents of rapid evolutionary change. Plasmids can carry bacterial accessory genes encoding diverse traits, such as metabolism of exotic substrates, colonisation of new habitats, or resistance to environmental toxins (Carattoli 2013, Frost et al 2005). The ability of plasmids to spread these traits to novel hosts can have important consequences for ecosystem and human health, demonstrated most strikingly in the spread by plasmids of antimicrobial resistance genes (Sheppard et al 2016). However, plasmid persistence in bacterial populations can be hard to explain. Not all plasmids carry accessory genes ('cryptic' plasmids (Zaleski et al 2015)), and even those that do are likely to be beneficial to their hosts only in specific environmental contexts (Gullberg et al 2014, Hall et al 2015). Moreover, experimental studies have shown that when they are

acquired, plasmids tend to levy a fitness cost on their hosts, meaning that plasmid-free competitors can outcompete plasmid-containing cells, driving plasmid extinction from the population (Dahlberg and Chao 2003, De Gelder et al 2007, Hall et al 2016). Yet plasmids remain widespread.

Different processes, mechanisms and interactions have been proposed to explain the long-term maintenance of plasmids. Positive selection for beneficial plasmid-borne accessory genes can maintain plasmids in the short term (De Gelder et al 2008). However, positive selection alone is unlikely to underlie prolonged plasmid maintenance because accessory genes are usually able to recombine with and become 'captured' by the chromosome, leaving the costly autonomous plasmid redundant (Hall et al 2016, Harrison and Brockhurst 2012). Theoretical studies have demonstrated that more complex environments may help to maintain the linkage between plasmids and the bacterial accessory traits they encode, for example by allowing transfer of locally-adaptive genes to immigrating genotypes (Bergstrom et al 2000), or lowering the relative cost of a public good trait by spreading that cost infectiously (Rankin et al., 2011). However, positive selection is unable to explain the abundance of cryptic plasmids that carry no beneficial genes. Likewise, active partitioning mechanisms (*par* modules) (Sengupta and Austin 2011) or the acquisition of toxin-antitoxin (TA) 'addiction' modules by plasmids (Loftie-Eaton et al 2016) can stabilise plasmids by reducing the rate at which plasmid free competitors arise. But these mechanisms alone cannot explain long-term survival because segregational loss is not reduced to zero and TA systems can be lost or moved onto the chromosome meaning plasmid-free individuals can emerge and benefit from growth advantage.

Given that positive selection is unable to explain the long-term stability of costly plasmids in the face of appreciable rates of segregational loss, two key processes have emerged that enable plasmid stability: infectious transmission and compensatory evolution.

Plasmids could counter the negative demographic effects of purifying selection and segregation by increasing their rate of infectious horizontal transmission into plasmid-free hosts, i.e. by becoming infectious agents. While this route is clearly unavailable to plasmids that do not encode their own conjugative apparatus, i.e. mobilizable plasmids (those that utilise the conjugation machinery of other elements) and non-mobilizable plasmids, a considerable proportion of large plasmids do carry their own conjugation apparatus (Smillie et al 2010). Among

these conjugative plasmids this process is typically tightly regulated by the plasmid itself via a complex gene-regulatory network (Bañuelos-Vazquez et al., 2017). These networks are often conservative, actively repressing plasmid transfer unless specific environmental, physiological or demographic conditions are met (Koraimann and Wagner 2014). This self restraint is likely to reflect the high cost of initiating conjugation for the bacterial host, which requires an investment of time and resources into building the conjugative pilus, and replication and transfer of DNA, resulting various physiological stresses (Zahrl et al 2006). Moreover, pili production can increase susceptibility of the host to environmental stressors (Bidlack and Silverman, 2004)as well as predation from lytic bacteriophages (Jalasvuori et al 2011). Among bacteria-plasmid relationships studied under laboratory conditions rates of conjugation in bacterial populations are often considered too low to explain plasmid stability (Bergstrom et al 2000), although various authors have argued that conjugation rates observed in lab media underestimate rates in natural environments (Lilley and Bailey 1997). However, experimental evolution has revealed that selection for horizontal transmission can lead to the evolution of increased conjugation rate in plasmids (De Gelder et al 2008, Kottara et al 2016, Turner et al 1998) and several studies have found plasmids with conjugation rates sufficient to allow them to invade and persist as infectious elements (Bahl et al 2007, Fox et al 2008, Hall et al 2016).

A number of long-term experimental evolution studies have now demonstrated that plasmid stability can be facilitated by compensatory evolution to ameliorate the cost of plasmid carriage thereby weakening purifying selection against the plasmid backbone (Dionisio et al 2005, Harrison et al 2015a, Heuer et al 2007, Porse et al 2016, San Millan et al 2015, Sota et al 2010, Zhong et al 2012). Moreover, signatures of plasmid amelioration can be detected by comparative genomics (McNally et al 2016). The experimental studies reveal that compensatory mutations ameliorating the plasmid cost can occur both on the plasmid (Dionisio et al 2005, Porse et al 2016, Sota et al 2010) or on the host chromosome (Harrison et al 2015a, San Millan et al 2015). Several known mechanisms of compensatory evolution involve mutations to regulatory genes which are likely to undo the gene regulatory disruption caused by plasmid acquisition, which is emerging as a common basis for the costs of newly acquired plasmids (Harrison et al 2015a, San Millan et al 2015). In some cases, single compensatory mutations in regulatory genes have been shown to completely ameliorate the cost of plasmid carriage (Harrison et al 2015a). In other cases, compensatory mutations have been shown to target conjugation leading to reduced

rates either indirectly, presumably as a result of large-scale regulatory changes (Harrison et al 2015a), or directly via mutations affecting genes involved in the conjugation process (Heuer et al 2007).

Thus increased rates of infectious transmission via higher rates of conjugation and amelioration to reduce the cost of plasmid carriage have both been shown to promote plasmid stability. However, there has been little consideration of how these two processes interact. As mentioned above, compensatory mutations can directly lead to reduced rates or complete loss of conjugation, likely due to the fact that conjugation itself is an inherently costly process. Experimental evolution studies provide contradictory evidence for how amelioration and infectious transmission interact. Several studies have reported evidence for a trade-off between increased plasmid conjugation rate and bacterial host fitness, suggesting that the physiological costs of conjugation scale with the rate of conjugation (Dahlberg and Chao 2003, Heuer et al 2007, Turner et al 1998). However, another study observed no such trade-off: plasmids that evolved higher conjugation rates also evolved to become less costly (Kottara et al 2016), suggesting that infectious transmission and amelioration need not always be mutually exclusive mechanisms for plasmid stability. There is a clear need for evolutionary theory to understand how these two key mechanisms of plasmid stability interact.

To expand on our verbal model and further explore the relationship between amelioration and infectious transmission in plasmid stability we developed an individual-based model of plasmid evolution (Harrison et al 2015b, Harrison et al 2016). Individual based models are a powerful tool to explore the dynamics of evolving systems, as they are stochastic and thus can be used to investigate the impact of evolutionary events, like rare mutations, on ecological processes, like plasmid dynamics. They are therefore useful for unpicking the potential and relative importance of different evolutionary processes and trajectories. We used our model to explore systems in which conjugation rate could evolve, amelioration could evolve, or both processes could evolve. We ran the simulation with varying rates and degrees of amelioration, and also explored the differences in plasmid maintenance between models in which amelioration mutations occurred on the plasmid compared with chromosomal mutations.

The model

Here we elaborate on a model parameterised to reflect the characteristics of a costly conjugative plasmid (Harrison et al 2015b, Harrison et al 2016). A full description of the model and parameter values can be found in the supplementary material. In brief, populations are modelled in continuous time (Allen and Dytham, 2009) with individuals randomly chosen and subjected to one of three possible events, chosen at random; cell division, conjugation, cell death. Whether or not the event occurs is dependant on a given probability (supplementary materials). If it does, the changes are recorded, but regardless of whether the event occurs or not, time is advanced, with time increments scaled by population size. We divide time here in to generations, which as it is continuous is somewhat arbitrarily, but amounts to roughly 3*n* events, at which point analytics are recorded. To simulate environmental disruption every 8 generations the population experiences a 1% bottleneck, where 99% of the population, drawn at random, dies.

Compensatory evolution

The mechanisms which underlie compensatory evolution are only starting to be understood but empirical evidence suggests that amelioration of plasmid cost is often accomplished through highly repeatable, large effect mutations (Harrison et al., 2015; Porse et al., 2016; San Millan et al., 2015) that can be very specific to different bacteria - plasmid combinations (San Millan et al., 2015). As we have previously shown both the strength and availability of these mutations can have important consequences for the fate of the conjugative plasmids (Harrison et al., 2016). Following our previous model the cost of plasmid carriage can be ameliorated in a single mutational step whereby either 100%, 95% or 50% of the cost of the plasmid is ameliorated. Compensatory mutations occur at the same (10^{-7}) or elevated (10^{-6} and 10^{-5}) mutation rate relative to the rest of the genome.

Further to this we allow mutations to occur on either the plasmid, i.e. remain linked to the plasmid following horizontal gene transfer, or on the bacterial genome, where naive hosts will be subject to the full cost of plasmid carriage.

Conjugation

Conjugation events are dependant on two probabilities; firstly the probability that a cell is in contact with another cell in the environment, which scales with the population size/density. If contact occurs - and the focal individual carries a plasmid - conjugation will be initiated with a given probability, here referred to as the conjugation rate, which is encoded by the plasmid. Plasmid encoded conjugation rates are free to evolve with mutational effects drawn at random. In order to capture the tradeoff between vertical and horizontal transmission, the next time that individual is selected for a cell division event the probability of cell division occurring is reduced to zero. Superinfection is not permitted meaning that attempted conjugation with a plasmid containing cell results in a growth rate cost but no transfer of the plasmid.

Analysis

Parameter effects on plasmid survival - i.e. the time to plasmid extinction with censoring to account for replicates where plasmids were maintained - was estimated where possible using the Cox proportional hazard method using the 'coxph' function in the 'survival' R package. As statistical analysis of data obtained through simulations is problematic, in particular due to inflated risk of type I error (White et al., 2014) effect size (exp(coeff)) but not p values are reported.

Results

Under the conditions of the model plasmids are lost from bacterial populations when conjugation rates or amelioration is allowed to evolve (Fig 1A). Both the evolution of increased conjugation rate and the emergence of amelioration could result in plasmid maintenance. Plasmid survival in each case was stochastic, with plasmids either persisting, often at high frequency, or being lost from the population entirely depending on the mutations arising before the plasmid went extinct; a situation akin to evolutionary rescue.

For models in which conjugation, but not amelioration, could evolve, plasmids survived in 10/60 replicates (Fig. 1A) and rose to high frequency. However, this result was highly dependent on mutations that increase plasmid conjugation rate arising before the plasmid became extinct. This outcome results in purely parasitic infectious plasmids that offset their cost of carriage by evolving a high rate of (re-)infection. Furthermore, plasmids maintained by high conjugation rates encounter an additional constraint due to the tradeoff between horizontal

and vertical transmission. This cost is captured in our model, as conjugation is associated with a reduction in growth. The benefits of horizontal vs vertical transmission will be exacerbated under conditions where horizontal transmission is inefficient, i.e. where conjugation does not result in plasmid infection, which may also be the case where the plasmid is at high frequency but also where potential recipients contain a different plasmid from the same incompatibility group or cellular immune systems such as CRISPR/cas loci. The result of this is that investment in conjugation will be frequency-dependent, leading to large oscillations in conjugation rate before reaching an equilibrium at an intermediate rate (Fig. 2B and C).

For models in which amelioration, but not conjugation, could evolve, plasmid maintenance was strongly dependant on the strength of amelioration mutations (% cost ameliorated: exp(coeff) = 0.751, SE(coeff) = 0.020), as well as on the mutation rate (log[amelioration mutation rate]: exp(coeff) = 0.689, SE(coeff) = 0.043) (Fig.1B). This is consistent with the stochastic nature of individual-based models: if survival depends on the mutation appearing before the plasmid was driven extinct, increased rates of mutation can have a large effect on the outcome. Variable survival was seen in models where amelioration occurred less frequently, provided the strength of amelioration was sufficient to tip the balance between loss though purifying selection and gain through conjugation, allowing plasmids to reinvade. The models suggested that plasmid-borne amelioration is more effective than host amelioration (amelioration location: exp(coeff) = 0.441, SE(coeff) = 0.155). This is because for plasmid amelioration, the plasmid and the amelioration mutation are linked, allowing them to spread together by both vertical and horizontal transmission, whereas for host amelioration reduced-cost plasmids are only transmitted vertically (i.e. to daughter cells). However, although few cases of amelioration have been conclusively identified in experimental studies, those that have been published suggest that host amelioration occur perhaps more readily than plasmid-borne amelioration (Harrison et al 2015a, San Millan et al 2015) but see (Porse et al 2016, Sota et al 2010). This may be due to the fact that there are potentially more chromosomal targets for amelioration — chromosomes are bigger than plasmids (often an order of magnitude or more), thus increasing the effective supply of ameliorative mutations. In addition, plasmid-borne ameliorations may be host specific, and therefore this advantage may be limited in situations where plasmids are transferred between host genotypes or species (Sota et al 2010).

For models in which both conjugation rate and amelioration were allowed to evolve, the mechanisms interacted synergistically to enhance plasmid maintenance (Fig. 1C. effect of conjugation evolution on amelioration models: exp(coeff) = 0.574, SE(coeff) = 0.112). This is because the evolution of either mechanism increases the likelihood of the other becoming established. Where amelioration evolved first plasmids could be maintained, giving time for high conjugation rates to evolve and spread plasmids throughout the population (Fig. 3A & B). Correspondingly, the early appearance of high conjugation rates increases plasmid prevalence and allows compensatory mutations to reach fixation (Fig. 3C & D), whereas alone they are only able to invade the plasmid containing portion of the population. In the long-term however, amelioration is predicted to be a more successful strategy than high conjugation rate. While high conjugation rates can drive plasmid invasion, once the population has ameliorated plasmid cost the need for high rates of conjugation are reduced. Under the conditions of our model, where plasmids are at high frequency and conjugation is inefficient there is a benefit to investing less in horizontal transmission and plasmid conjugation rates decrease (Fig 3B & D). Interestingly, some plasmid-borne accessory genes confer benefits which scale negatively with plasmid frequency in the population (Ellis et al 2007), indicating additional costs to conjugative plasmids in populations where they are at high frequency.

Our results suggest that established bacteria-plasmid relationships should thus ultimately trend towards low conjugation rate, because high conjugation rate is only transiently beneficial. For many, possibly all, natural plasmids, conjugation is tightly controlled through a plasmid encoded gene-regulatory network which represses plasmid transfer gene expression unless specific environmental, physiological or demographic conditions are met (Koraimann and Wagner 2014). This self restraint is likely to reflect the high cost of conjugation initiation, limiting conjugation to conditions where plasmid transfer is more likely to be successful, e.g. where bacterial population density is high (McAnulla et al., 2007), or beneficial e.g. when hosts are under physiological stress (Beaber et al., 2003). Whilst here we assume that conjugation is subject to regulation based on bacterial density, the mechanism underlying conjugation regulation may well have an impact on the predictions of the model. This is particularly true for mechanisms that restrict conjugation under conditions where plasmid-free recipients are likely to be rare. This may be achieved indirectly, through transient derepression systems, where the infectivity of newly acquired plasmids is initially high but repression builds up over time. At the population level this will allow conjugation rates to be high where new hosts are abundant and naturally decline as the plasmids become

saturated in the population (Fernandez-Lopez et al 2014). In addition more direct mechanisms have been identified which use either plasmid (Singh et al., 2013) or host encoded pheromones (Dunny and Johnson, 2011) to detect the frequency of plasmid free recipients and therefore repress conjugation as plasmids approach saturation. Such mechanisms circumvent the frequency-dependant benefits that are inherent in plasmid conjugation and lead to selection against highly infectious plasmids in our model.

In contrast to increased conjugation rate, amelioration was universally favoured and does not suffer a penalty at high plasmid frequency. Thus whilst high conjugation rates can facilitate the invasion of plasmids into new bacterial hosts, where compensatory mutations are available these mutations will inevitably spread, reducing purifying selection which drives plasmid loss and thus selection for high conjugation rates. Over evolutionary timescales therefore, evolution to ameliorate plasmid costs is likely the primary mechanism enabling plasmid persistence. Moreover, our simulations suggest that plasmid amelioration is a more effective evolutionary solution than is host amelioration, although this is likely to vary with ecological conditions. For example, the relative benefits of plasmid versus host amelioration may depend on the balance of vertical versus horizontal transmission, with host amelioration likely to be more favoured with diminishing opportunities for horizontal transmission.

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Figure 1. Overview of plasmid prevalence across the model parameter space. Circles denote the outcome of replicate simulations for each set of model parameters. Each is composed of 20 sectors, showing the plasmid prevalence (height, where the circle center = 0 and outline = 1) mean conjugation rate (shading) for a single replicate simulation after 1000 generations. A. Models where no compensatory evolution is permitted. For these treatments 60 replicate simulations were run (split across 3 circles, showing 20 replicates per circle). B. Models

where amelioration but not conjugation was allowed to evolve. The effect size (x axis) and mutation rate (y axis) of available amelioration mutations was varied, as well as the position on either the plasmid or chromosome. C. Models where both conjugation rares and amelioration was allowed to evolve. Axes as for B.

Figure 2. Rescue of plasmids through conjugation rate evolution. Population dynamics of a single iteration of the model where conjugation rate evolution occurs in time to prevent plasmid loss in the absence of amelioration mutations. Panels on the left show A. plasmid prevalence (shading) and B. corresponding plasmid conjugation (shading: mean conjugation rate; line: number of conjugation events per plasmid) through time. Panel C. Oscillations in conjugation rate are driven by plasmid-frequency dependant selection stabilised over time (shown as line shading from 0 (light) to 1000 (dark) generations).

Figure 3. Examples of the synergistic effect of combined conjugation and amelioration evolution in stabilising plasmid prevalence. Panels A and C show plasmid prevalence (light shading) and the frequency of ameliorated plasmid genotypes (dark shading) and panels B and D show corresponding plasmid conjugation (shading: mean conjugation rate; line: number of conjugation events per plasmid) through time. In iteration 1 conjugation rate evolves first allowing the plasmid to invade to high frequency. As a consequence ameliorated plasmid bearers are able to invade into the plasmid containing portion of the population. In iteration 2 amelioration mutations stabilise plasmid prevalence at low frequencies, allowing time for conjugation rate mutations to appear before the plasmid is lost. Both iterations are examples of dynamics from models where conjugation evolves, amelioration occurs on the chromosome, efficiency = 100% and mutation rate = $5e10^{7}$.

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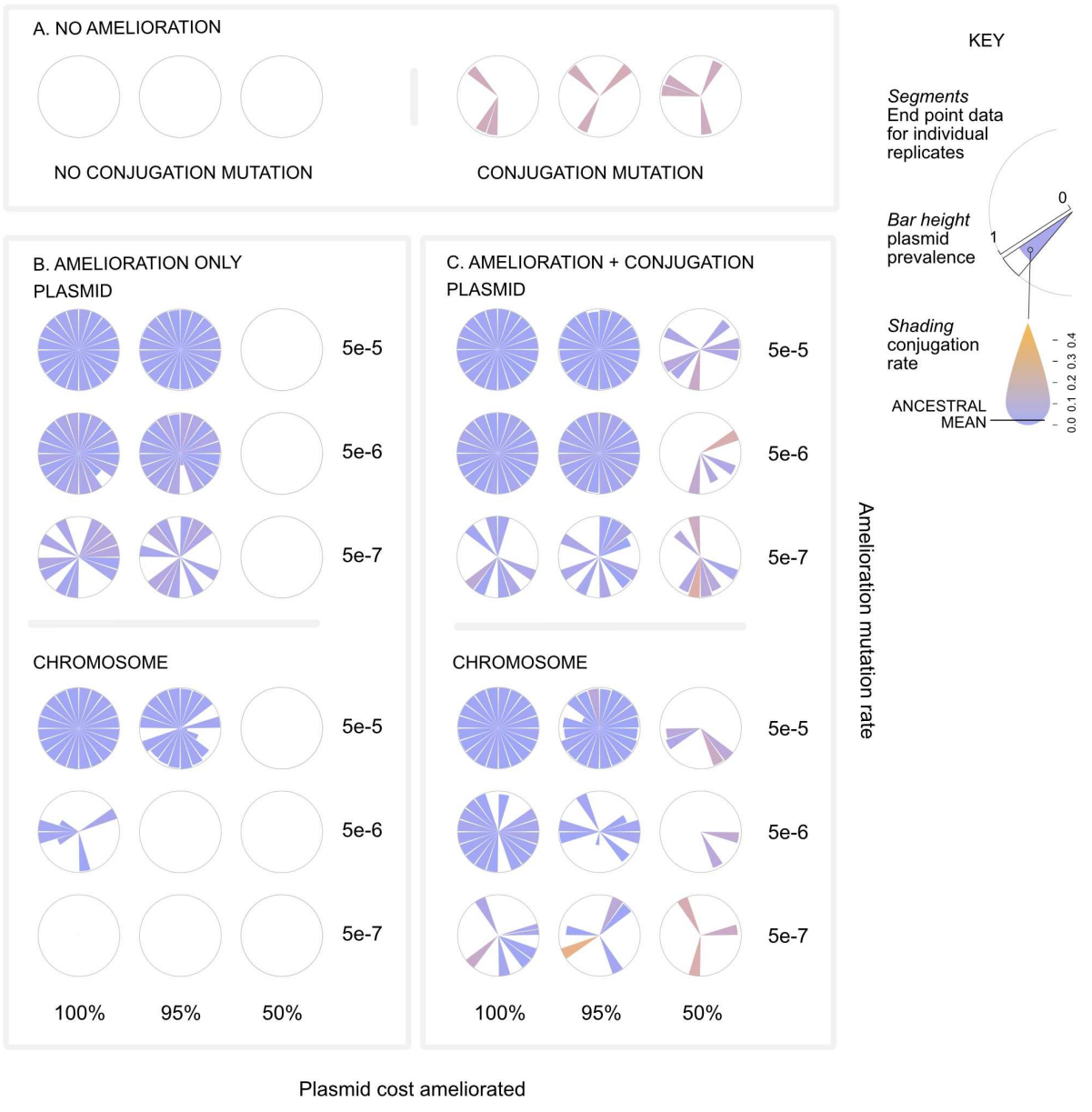
A. NO AMELIORATION
NO CONJUGATION MUTATION
CONJUGATION MUTATION
B. AMELIORATION ONLY
PLASMID
5e-5
5e-6
5e-7
CHROMOSOME
5e-5
5e-6
5e-7
100%
95%
50%
C. AMELIORATION + CONJUGATION
PLASMID
5e-5
5e-6
5e-7
CHROMOSOME
5e-5
5e-6
5e-7
100%
95%
50%
KEY
Segments
End point data
for individual
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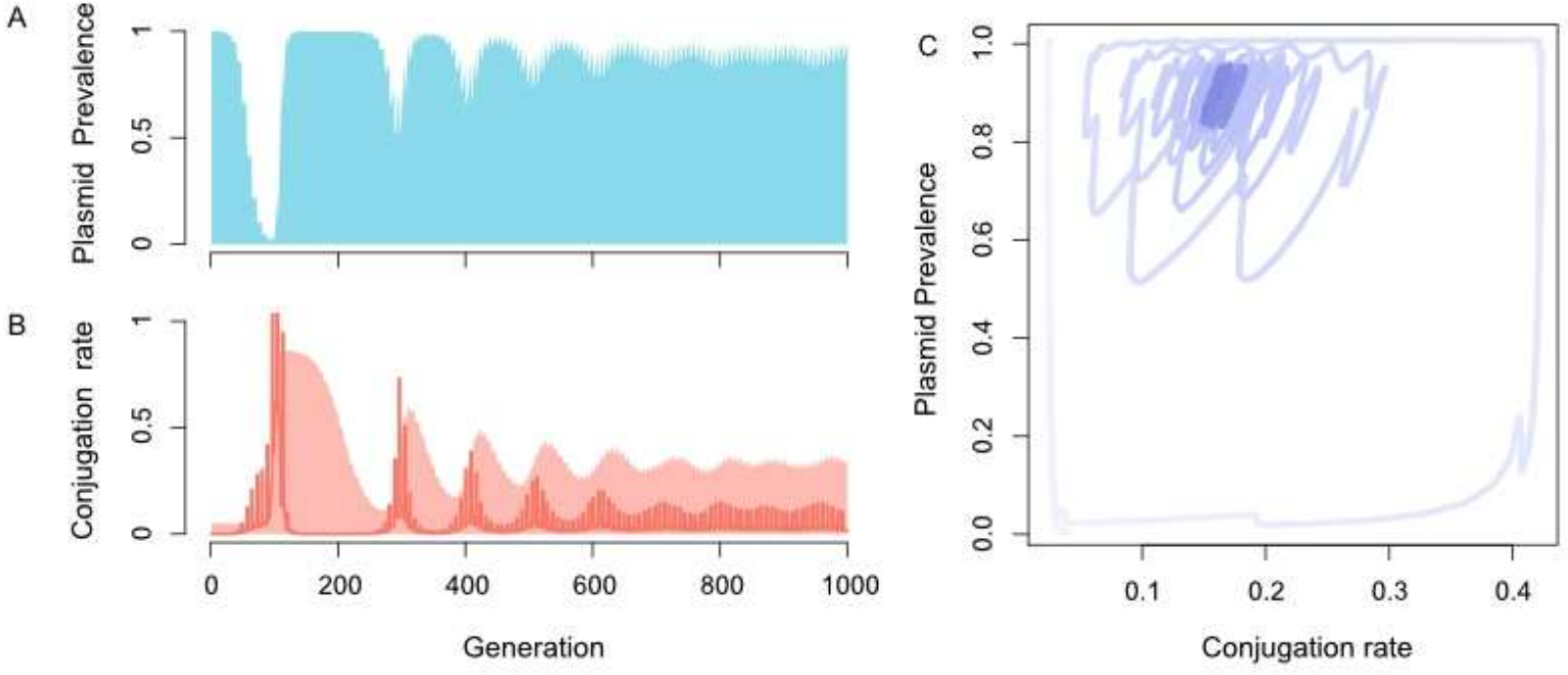
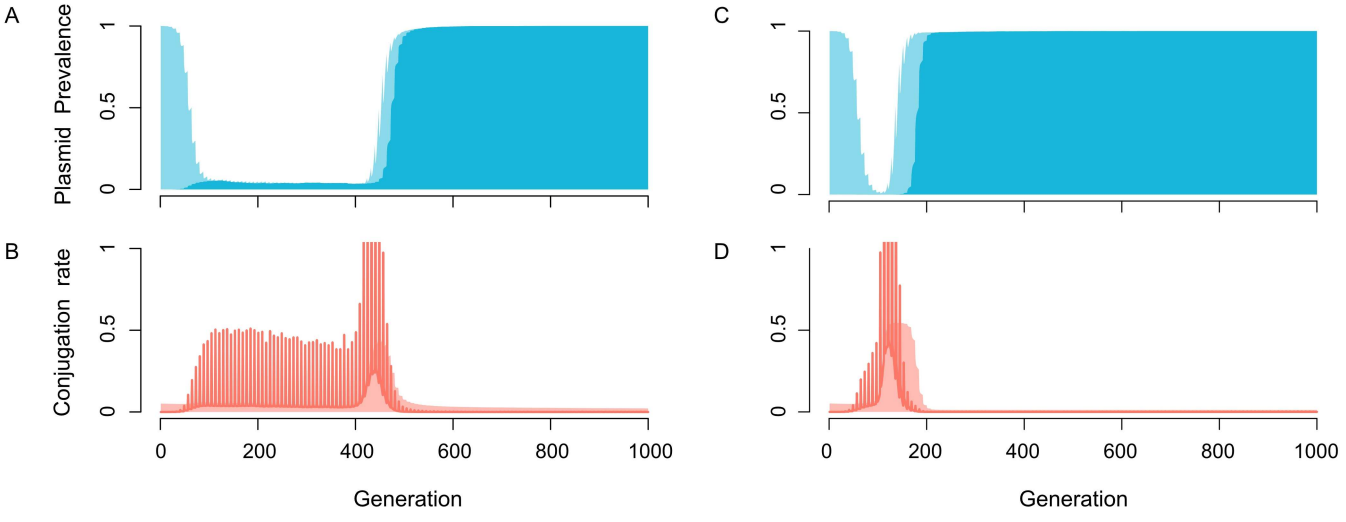
plasmid
prevalence
0
1
Shading
conjugation
rate
ANCESTRAL
MEAN
0.0 0.1 0.2 0.3 0.4
Amelioration mutation rate
Plasmid cost ameliorated


A
Plasmid Prevalence
0
0.5
1
B
Conjugation rate
0
0.5
1
0
200
400
600
800
1000
Generation
C
Plasmid Prevalence
0.0
0.2
0.4
0.6
0.8
1.0
0.1
0.2
0.3
0.4
Conjugation rate


A
Plasmid Prevalence
0
0.5
1
C
0
0.5
1
B
Conjugation rate
0
0.5
1
D
0
0.5
1
0
200
400
600
800
1000
Generation
0
200
400
600
800
1000
Generation

Supplementary information

Individual Based Model methodology

We developed an individual-based model using single bacterial cells as unique agents. Simulations are all initiated with 2,000,000 cells, initially 100% plasmid bearing, and are run for 1000 time steps. Populations are allowed to grow to a carrying capacity (*K*) of 5,000,000 with the probability of cell division scaled with population density, declining to 0 at *K*. We vary parameters for amelioration (the mutation rate, strength of amelioration mutations and their linkage to/location on either the plasmid or chromosome) and follow the fate of plasmids in the population with conjugation rate is either fixed or allowed to evolve (see table for parameter ranges). This gives 38 different conditions, and we have 20 replicates of each condition – with the exception of the no amelioration conditions (conjugation with and without conjugation evolution for which 60 replicates were run.

The model follows the rejection method of Allen and Dytham (2009) where events occur in series. Cells are selected at random and subject to one of three possible events in the model chosen at random: cell division; cell death; conjugation. We calculate the probability of that event occurring and either execute the event and update status or do nothing. Time then advances, whether the event is executed or not, by an amount drawn from an exponential distribution with mean 1/3n where n is the current population size (i.e. the number of events per unit time (here termed a 'generation') is proportional to the population size and a cell surviving an entire time step will experience an average of 1 event of each type during that time step).

When a generation is completed various statistics are collected and recorded. No status is changed at the end of the generation however to capture the stochasticity introduced through population disruption and bottlenecking (inherent in transfer experiments as well as naturally disturbed populations) every 8 generations the population is subject to 99% mortality.

Events

Cell division is density dependent with probability declining to zero at the carrying capacity. Any costs, such as the cost of carrying a plasmid, are applied as a reduction in the probability of cell division. Daughter cells are identical to the parent cell, except for 1) in plasmid bearers, the possibility of losing the plasmid through segregation, 2) the possibility of mutating the conjugation rate and, 3) for cells that have not ameliorated the cost of

bearing a plasmid, the possibility of mutating to allow amelioration of the cost of plasmid carriage.

Cell death is not density dependent and remains at a fixed rate throughout.

Conjugation is the only event type that requires direct interaction between cells. A conjugation event is initiated when the focal cell is plasmid bearing. First, we assume a well-mixed population and the focal cell encounters another random cell with probability 2n/K (where n is current population size and K is the carrying capacity). If an encounter occurs conjugation is initiated by the donor with a probability set by the donor cell's conjugation rate. If the encountered cell is plasmid free conjugation is successful and the encountered cell gains a plasmid. If it is plasmid-containing no transfer occurs. Donor cells pay a cost for the initiation of a conjugation attempt, regardless of outcome, by missing its next cell division opportunity.

Parameters

This model extends the simulation model described previously (Harrison et al., 2016, 2015b). Parameter estimates are drawn from experimental data based on the Pseudomonas plasmid pQBR103 (Harrison et al., 2015a) where possible as well as the literature.

Parameter	**Applied as**	**Value**
Population		
Starting population		2,000,000
Carrying capacity (*K*)		5,000,000
Mortality at bottleneck death rate		0.99
Bottleneck frequency		8 generations
Cell division event		
Birth rate	Cell division probability	Variable (n/*K)* where n is the current population size
Cost of plasmid carriage	Reduction in cell division probability	0.2[1]
Segregation rate	Probability of generating a plasmid-plasmid free daughter cell if plasmid containing	0.0001[2]
Mutation rate amelioration	Probability of daughter cells acquiring amelioration mutation	Variable ($5*10^{-5}$, $5*10^{-6}$, $5*10^{-7}$) [1]

Strength of amelioration mutations	Ameliorated cost of maintaining a plasmid	Variable (0, 0.01, 0.1) [1]
Mutation rate conjugation	Probability of plasmid in daughter cell acquiring conjugation mutation	$1*10^{-6}$
Strength of conjugation mutations	Change to probability of conjugation	Variable (Drawn from a random distribution with mean 0 and SD 0.2) NB. conjugation is capped at 0 and 1
Cell death event		
Death rate	Probability of cell death	0.1
Conjugation event		
Encounter rate	Probability of encountering another cell for possible conjugation	2 * n/*K* where n is the current population size Capped at 1
Starting conjugation rate	Probability of initiating conjugation if encounter occurs (prior to conjugation evolution)	0.025 [1]

[1] Parameterised based on experimental data from Pseudomonas plasmid pQBR103 (Harrison et al., 2015a)
[2] Estimate based on segregation rate of Pseudomonas plasmid pWWO (Duetz and Van Andel, 1991)

Allen, G.E., Dytham, C., 2009. An efficient method for stochastic simulation of biological populations in continuous time. Biosystems. 98, 37–42.

Duetz, W., Van Andel, J.G., 1991. Stability of TOL plasmid pWWO in Pseudomonas putida mt-2 under non-selective conditions in continuous culture. J. Gen. Microbiol. 137, 1369–1374.

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Brockhurst, M.A., 2015b. Bacteriophages limit the existence conditions for conjugative plasmids. MBio 6, e00586.